Cumhuriyet Dental Journal: 2020; 23(2)
e-ISSN 2146-2852
Doi:10.7126/cumudj. 704107

Original research

APICAL SEALING ABILITY OF DIFFERENT ENDODONTIC SEALERS USING GLUCOSE PENETRATION TEST: A STANDARDIZED METHODOLOGICAL APPROACH

ABSTRACT

Objectives: To compare the apical sealing ability of four endodontic sealers based on glucose penetration method and validate the uses of contralateral teeth to provide a well-balanced experimental group.

Materials and methods: One-hundred-and-twenty (sixty pair) extracted contralateral lower premolars were selected and undergonestrict radiographic protocol. Root canal anatomy of each pair contralateral teeth was matched buccolingually and mesiodistally according to inclusion criteria (single canal, mature apical foramen, canal type, canal width, length, and curvature). Matched-pair contralateral teeth were then reevaluated using CBCT and divided into right and left sides (n=60, each side). Next, all canals were instrumented up to size 30, taper 0.06. Subsequently, teeth were subdivided into five groups for each sideand obturated with single cone gutta-percha (GP) and various sealers: Group 1 - GP only (control); Group 2 - EndoRez; Group 3 - Sealapex; Group 4 - EndoSeal MTA and Group 5 - BioRoot RCS. All samples were placed in an incubator at 37°C, 100% humidity for 72 hours. Four matched-pair teeth from each group were then subjected to thermocycling for 100 cycles, 1000 cycles and 10000 cycles, respectively. After that, they were decoronated, coated with three layers of nail varnish, and used for glucose penetration test. The concentrations of glucose (mmol/L) were measured after 24 hours. Data analyzed using One-way ANOVA complemented by post hoc Dunnett T3 Test and Paired sample T-Test.

Results: EndoSeal MTA demonstrated statistically significant (p<0.05) lowest glucose penetration followed by BioRoot RCS, Sealapex, EndoRez, and lastly control group. Apical sealing ability decreased as the number of thermocycles increased. No significant difference (p>0.05) was found between matched-pair contralateral teeth.

Conclusions: Bioceramic sealers demonstrated better sealing abilitythan resin and calcium hydroxide sealers. Using matched-pair contralateral teeth provided a well-balanced experimental group.

Keywords: EndoRez, mineral trioxide aggregate, root canal filling materials, sealapex, tricalcium silicate.

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Received : 01.04.2020
Accepted : 30.04.2020

How to Cite: Lin G.S.S., Abdul Ghani N.R.N., Noorani T.Y., Kamarudin A. Apical Sealing Ability of Different Endodontic Sealers Using Glucose Penetration Test: A Standardized Methodological Approach. Cumhuriyet Dent J 2020;23:2;79-87.
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INTRODUCTION

Endodontic treatment involves the removal of pulp and cleaning of the root canal system to preserve the tooth in the dental arch.[1] This treatment is reported to have a high success rate range between 86–98%.[2] Recently, great attention has shifted towards the seal of the root canal system, as adequate obturation of the prepared three-dimensional root canals is important in determining the long term success of endodontic treatment.[3,4] With the use of gutta-percha and endodontic sealers, obturation allows hermetic seal of the canals, thus, prevents bacterial micro-leakage into the canals and provides good long-term prognosis.[5,6]

In the past few decades, numerous endodontic sealers have been introduced and they are classified based on their main constituent, for instance, resin, calcium hydroxide, glass ionomer, and mineral trioxide aggregate (MTA) sealers.[7] The introduction of adhesive dentistry concept allowed materials to bond and provide intimate contact with the dentine walls of the root canal.[8] Bondable root canal sealer, such as methacrylate resin sealer can form a monoblock system within the root canal space which improves the seal and fracture resistance of the filled canals.[8, 9] Recently, bioceramics have become one of the most popular biomaterials used in endodontics after the clinical success of MTA.[10-12] BioRoot RCS, a tricalcium silicate-based material, is amongst the most recently introduced bioceramic based endodontic sealer in the market. Bioceramic sealer sexhibit several advantages such as lower cytotoxicity, excellent antimicrobial activity due to its high pH value, promotes hard tissue formation and can form hydroxyapatite layer.[13]

Undeniably, the sealing ability of an endodontic sealer is still considered animportant parameter to be evaluated, but this assessment has been despised due to the lack of standardization.[7] There are a substantial number of studies among the literature that have claimed to evaluate the quality of seal of different endodontic sealers using an array of methods.[5,7,14-17] However, there is still no clear answer on the appropriateness of these leakage methodologies with questionable scientific significance. The reliability of leakage studies remains unclear and most of them are non-reproducible.[18] Therefore, a well-controlled condition is needed for assessing and comparing the sealing ability of endodontic sealers.

Hence, the present study aimed to compare the sealing ability of resin, calcium hydroxide and bioceramic endodontic sealers to root dentinal walls of endodontically treated teeth after artificial ageing using glucose penetration method.[19] Furthermore, the present study also aimed tovalidate the use of matched-pair contralateral teeth in providing a well-balanced experimental group for leakage study. The first null hypothesis tested was that there was no significant difference in terms of sealing ability among all four endodontic sealers. The second null hypothesis was there is no significant difference between the results of glucose penetration when comparing each matched-pair contralateral teeth used in this study.

MATERIALS AND METHODS

This was an *in-vitro* experimental study involving one-hundred and twenty (sixty pairs) human contralateral lower premolars recently extracted due to orthodontic reasons from patients of Asian origin and patients' age ranging from 20 to 40 years who attended dental clinics of Hospital Universiti Sains Malaysia. Ethical approval was obtained from the Human Research Ethics Committee USM (Ref. USM/JEPeM/18110691) on 10th January 2019. All teeth were inspected under Leica microscope (Leica Microsystem Imaging Solutions, Cambridge, UK) at a 20x magnification by two blinded examiners to ensure that they were free from fracture, abrasion, resorption defect, and root caries. The tooth length was measured using a metal ruler (CLR6, Hu-Friedy Mfg. Co. Inc., Chicago, USA) to include teeth with a total length of 21mm to 23mm and root length of 12mm to 14mm. Strict screening protocol with a digital radiographic examination (PlanmecaRomexis®, Helsinki, Finland) was then carried out by matching the root canal anatomy of eachpair contralateral teeth both buccolingually (BL) and mesiodistally (MD) to provide a consistent baseline. Only contralateral teeth with single canal, mature apical foramen, Type 1 Vertucci's Classification, anatomical root canal width difference of ±0.5mm, canal length difference of ±1mm (measuring from the cementoenamel-junction to apical foramen) and canal curvature difference (BL or MD) less than 25º were accepted for this study, whereas the remaining pairs of contralateral teeth were excluded. These step-by-step screening procedures were reevaluated again with three-dimensional (3D) radiographic analysis using Cone Beam Computer Tomography (CBCT) scan (Art 3D, Oy Ajat, Espoo, Finland) taken by a licensed radiologist and images taken were analyzed using Romexis 2.9.2 R software (PlanmecaRomexis®, Helsinki, Finland) to avoid selection mistake. Only sixty matched-pair contralateral lower premolars (n=120) were chosen after the selection process. Each matched pair contralateral teeth were then divided into the left side, α (n=60) and right side, β (n=60). They were numbered accordingly to ensure a well-controlled comparison for each matched-pair contralateral teeth. Soft tissue debris and calculus were removed using an ultrasonic scaler (Dentsply Sinora, Bensheim, Germany). Access cavities were then prepared using a diamond Endo-Access bur, 21mm, size 3 (A 0164, Dentsply Maillefer, Switzerland) and canal patency was checked using sizes 10 and 15 K-files (FlexOFiles; Dentsply Maillefer, Switzerland). Root canals were instrumented with NiTi rotary files (S5 Sendoline, Tillverkarvägen 6, SE-187 66 TÄBY, Sweden) up to the final size 30, 0.06 taper to the working length, 1 mm short from the radiographic apex. After that, canals were irrigated copiously using 2.5% sodium hypochlorite (Lenntech, Delfgauw, Netherlands) solution (NaOCl). Finally, 5ml of 17% ethylenediaminetetraacetic acid (EDTA) solution (Promega Corporation, Wisconsin, USA) was used to remove smear layer followed by another 5ml of normal saline solution (RMBIO, Missoula, Montana) as final irrigation to wash out remnants of EDTA in the root canals. The canals were dried with paper points size 30 (Dentsply, Maillefer, USA). Contralateral teeth were subdivided into five groups for each side and obturated with matched gutta-percha size 30 taper 0.06 (Meta Dental Corp, Glendale, New York, US) using single cone technique and various endodontic sealers as below:

Group 1: Gutta-percha only without sealer (control)

Group 2: EndoRez (Ultradent Products, Inc., South Jordan, US)

Group 3: Sealapex (Kerr Corporation, Orange, California, US)

Group 4: EndoSeal MTA (Maruchi, Gangwon-do, South Korea)

Group 5: BioRoot RCS (Saint-Maur-des-Fossés Cedex, France)

The sealers were mixed according to manufacturers' instructions. Sealers were first coated around the canal walls using the matched gutta-percha point before placing gutta-percha into the canal. All canals were eventually obturated using single cone technique with matched gutta-percha point and respective sealers. Excess gutta-percha was cut off and access cavities were cleaned after obturation. The coronal accesses were then acid etched (Gel Etchant, Kerr Corporation, Orange, CA) for 10 seconds and bonding agent (OptiBond™ Universal, Kerr Corporation, Orange, CA) applied followed by light curing for 15 seconds and restored with microhybrid resin composite (Zmack, Italy) incrementally with adequate 40 seconds of light-curing using a pre-calibrated LED light-curing unit Elipar Free Light 2 (3M ESPE, St. Paul, MN, USA) with a light intensity of 800 mW/cm^2. Final composite restorations were polished with composite polishing kits (PN 0310BB, Composite Polishing Kit CA, Shofu, CA, US). The teeth were then placed in an incubator (ICS200, Yamato Scientific Co., Ltd., Japan) at 37°C, 100% humidity for 72 hours to allow complete setting of the sealers. Four matched-pair teeth from each groupon both sides were randomly selected and subjected to 100 thermal cycles using a thermocycling machine (TS Series Liquid, Weiss Technik, North America) in sequential water baths of 5°C, 37°C and 55°C. The dwell time was set at 30 seconds with a transfer time of 5 seconds. The same thermal cycle process was repeated accordingly with the other four matched-pair teeth from each group for 1000 thermal cycles. Lastly, the remaining four matched-pair teeth from each group were subjected to 10000 thermal cycles. Teeth were kept moist throughout the experiment by covering them with moist gauze.

Glucose Penetration Test

After thermal cycles, the teeth were decoronatedat the cementoenamel junction (CEJ)using a hard tissue cutter (EXAKT 312, EXAKT Technologies, Inc., Oklahoma City, US).Only sample in the control group (Group 1) were subdivided into positive (n=12) and negative (n=12) controls, each consisted of 6 matched-pair teeth.[11] All samples were coated with three layers of nail varnish leaving 1mm clear from the apical foramen and 1mm clear from the CEJ, except samples in the negative control group were entirely coated with three layers of nail varnish to prevent the glucose molecules from leaking out through lateral and other accessory canals which might affect the validity of the present study. Then, samples were set up as shown in Figure 1.

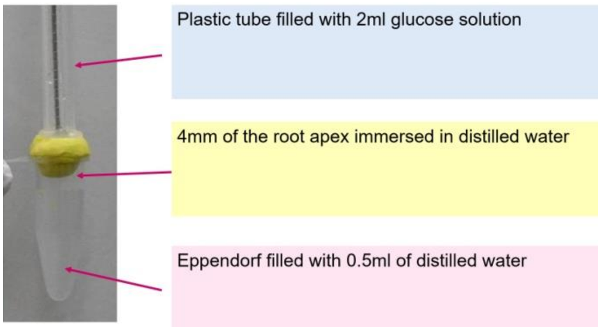

Figure 1: The set-up of experiment for glucose penetration test.

The samples were attached to the end of the plastic tube using sticky wax and glued to the opening of a 1.5ml Eppendorf (Eppendorf Asia Pacific Sdn. Bhd., Selangor, Malaysia). The Eppendorf was filled with 0.5ml of distilled water and only 4mm of the root apex was immersed into the distilled water. A 1mg/ml glucose solution (Standard glucose solution, Sigma Aldrich, USA) with a molecular weight of 180g/mol was used as tracer in this study.2ml of the glucose solution was injected into the plastic tube until it reached a height of 14cm to allow a hydrostatic pressure of 1.37kPa exerted on the gutta-percha and sealer. The samples were left for 24 hours in the same incubator at 37°C and 100% humidity to allow the glucose molecule to penetrate the root canals into the distilled water. The concentrations of glucose (mmol/L) in the distilled water were measured using glucose kit (Contour Plus, Ascensia Diabetes Care Holdings AG., Switzerland) and the data were recorded.

Statistical analysis

Data analysis for glucose penetration was carried out using SPSS version 24.0 for Windows (SPSS Inc., Chicago, IL, USA).One-way ANOVA complemented by post hoc Dunnett T3 Test were used for inter-group comparison with the significance level set at p=0.05.The differences in concentration of glucose penetration (mmol/L) for both matched pair contralateral teeth, α and β, were analyzed using Paired Samples T-test.

RESULTS

The results of glucose penetration are shown in Table 1. The samples used as controls behaved as expected in which the negative control groups showed no glucose penetration during the entire experiment, but positive controls exhibited the highest rate of glucose penetration leakage. A significant difference was noted (p<0.05) with the positive control in Group 1 showing the highest mean of glucose penetration, followed by EndoRez in Group 2, Sealapex in Group 3, BioRoot RCS in Group 5and lastly EndoSeal MTA in Group 4after 100, 1000 and 10000 thermocycles respectively. However, no significant difference was noted between Group 4 and Group 5after 100, 1000 and 10000 thermocycles (p=0240; 0.992; 0.979), respectively.

Table 1: Concentrations of glucose penetration (mmol/L) of different endodontic sealers using One-way ANOVA complemented by Dunnett T3 Test

Group	Type of Sealer	Mean (SD)	F(d*f*)	p-value	Multiple Comparisons Groups	Mean Diff.	Std. Err.	p-values
100 thermocycles								
1	Positive Control	5.40 (± 0.65)	313.17 (4, 25)	0.001*	1 vs 2	4.467	0.282	0.001*
					1 vs 3	4.917	0.269	0.001*
2	EndoRez	0.93 (± 0.24)			1 vs 4	5.335	0.265	0.001*
					1 vs 5	5.313	0.265	0.001*
3	Sealapex	0.48 (± 0.12)			2 vs 3	0.450	0.110	0.033*
					2 vs 4	0.868	0.099	0.002*
4	EndoSeal MTA	0.07 (± 0.01)			2 vs 5	0.847	0.099	0.003*
					3 vs 4	0.418	0.048	0.002*
5	BioRoot RCS	0.08 (± 0.01)			3 vs 5	0.397	0.048	0.003*
					4 vs 5	-0.022	0.005	0.240
1000 thermocycles								
1	Positive Control	8.55 (± 0.78)	470.15 (4, 25)	0.001*	1 vs 2	6.067	0.332	0.001*
					1 vs 3	7.133	0.324	0.001*
2	EndoRez	2.48 (± 0.23)			1 vs 4	7.950	0.323	0.001*
					1 vs 5	7.867	0.320	0.001*
3	Sealapex	1.42 (± 0.15)			2 vs 3	1.067	0.112	0.001*
					2 vs 4	1.883	0.111	0.001*
4	EndoSeal MTA	0.60 (± 0.14)			2 vs 5	1.800	0.099	0.001*
					3 vs 4	0.817	0.083	0.864
5	BioRoot RCS	0.61 (± 0.13)			3 vs 5	0.808	0.062	0.001*
					4 vs 5	-0.025	0.006	0.992
10000 thermocycles								
1	Positive Control	12.30 (± 0.38)	2221.25 (4, 25)	0.001*	1 vs 2	6.133	0.180	0.001*
					1 vs 3	9.117	0.114	0.001*
2	EndoRez	6.17 (± 0.22)			1 vs 4	11.333	0.180	0.001*
					1 vs 5	11.233	0.171	0.001*
3	Sealapex	3.18 (± 0.18)			2 vs 3	2.983	0.116	0.001*
					2 vs 4	5.200	0.125	0.001*
4	EndoSeal MTA	0.97 (± 0.22)			2 vs 5	5.100	0.111	0.001*
					3 vs 4	2.217	0.116	0.001*
5	BioRoot RCS	1.01 (± 0.16)			3 vs 5	2.117	0.110	0.121
					4 vs 5	-0.100	0.011	0.979

*Statistically significant

Results in Table 2 showed no significant difference ($p>0.05$) of glucose penetration when comparing both matched-pair contralateral teeth, α and β.

Table 2: Concentration of glucose penetration (mmol/L) of different endodontic sealers in both matched-pair contralateral teeth, α and β, using the Paired Samples T-test.

Group	Type of Sealer	Mean (SD) Left Tooth, α	Mean (SD) Right Tooth, β	Mean diff.	Std. Error	p-values
100 Thermocycles						
1	Positive Control	5.57 (± 0.81)	5.23 (± 0.57)	0.333	0.187	0.214
2	EndoRez	1.00 (± 0.26)	0.87 (± 0.25)	0.133	0.067	0.184
3	Sealapex	0.47 (± 0.15)	0.50 (± 0.10)	0.033	0.031	0.423
4	EndoSeal MTA	0.07 (± 0.02)	0.06 (± 0.01)	0.040	0.031	0.321
5	BioRoot RCS	0.09 (± 0.01)	0.08 (± 0.01)	0.007	0.003	0.284
1000 Thermocycles						
1	Positive Control	8.43 (± 1.11)	8.37 (± 0.45)	0.367	0.376	0.432
2	EndoRez	2.57 (± 0.21)	2.40 (± 0.26)	0.167	0.033	0.183
3	Sealapex	1.43 (± 0.15)	1.40 (± 0.17)	0.233	0.088	0.118
4	EndoSeal MTA	0.60 (± 0.20)	0.60 (± 0.10)	0.200	0.058	0.892
5	BioRoot RCS	0.59 (± 0.15)	0.63 (± 0.06)	0.067	0.013	0.423
10000 Thermocycles						
1	Positive Control	12.33 (± 0.45)	12.27 (± 0.41)	0.067	0.145	0.691
2	EndoRez	6.17 (± 0.31)	6.37 (± 0.12)	0.333	0.133	0.130
3	Sealapex	3.10 (± 0.20)	3.17 (± 0.15)	0.301	0.058	0.350
4	EndoSeal MTA	0.91 (± 0.10)	1.01 (± 0.31)	0.267	0.033	0.508
5	BioRoot RCS	1.03 (± 0.21)	1.00 (± 0.10)	0.133	0.067	0.184

*Statistically significant

DISCUSSION

The first null hypothesis was rejected because a significant difference was found between the sealing ability of endodontic sealers. In the current study, methacrylate resin-based sealer, EndoRez demonstrated the poorest sealing ability which is in agreement with other findings.[20-22] EndoRez, a second-generation bondable sealer is able to flow into accessory canals and dentinal tubules to promote the formation of resin tag for retention, but it was reported to exhibit low bond strength to the dentinal wall which could be one of the reasons of its poor seal.[8, 23] Another factor that attributed toits poor sealing ability is the intrinsic volumetric shrinkage and interfacial stress during polymerization that causes gap formation between the sealer material and dentine wall.[24] Additionally, the C-factor in a root canal is extremely high, which causes the sealer material to debond from dentine walls and causes microleakage due to improper seal.[25] Sealapex, a calcium hydroxide-based sealer, in the present study showed slightly better sealing ability than the resin sealer. Sealapex can forms chemical bond between isobutyl salicylate found in the material itself and calcium in the tooth structure that leads to better sealing and adaptation to root canal walls.[26] However, in the present study, Sealapex demonstrated poorer sealing ability than the other two bioceramic sealers which is in contradictory with several studies.[14-16] The difference in the results could probably due to the methodological design of different studies.

Bioceramic sealers have recently gained attention in the field of endodontic since they can form anapatite layer, allowing intrafibrillar apatite deposition.[13,27] This promotes the formation of a tag-like structure which plugs along with the dentine bonding interface, thus, creating a strong mineral infiltration zone resulting in a better seal.[28] Although bioceramic sealers (EndoSeal MTA and BioRoot RCS)in the present study demonstrated excellent sealing ability which is in agreement with the other authors[29-31], but several studies found that there is no significant difference when comparing the sealing ability of bioceramic sealers with resin and calcium hydroxide based sealers.[31-33]A recent study also reported that BioRoot RCS demonstrated a higher percentage of voids as compared to the conventional epoxy resin sealer, AH Plus.[7] Information regarding sealing ability of BioRoot RCS is still scarce and controversial in the literature, therefore, more studies need to be done on the sealing ability of this new bioceramic sealer to provide a better comparison.

Based on the results of the present study, all sealers showed a decrease in sealing ability as the number of thermocycle increased. Thermocycling process was used to simulate and accelerate the physiological ageing of materials in clinical setting.[34] Thermal test tend to stress the bond between the materials by causing continuous expansion and contraction, thus, resulting in crack propagation and gap formation.[35] However, the use of thermocycling for endodontic sealers remains controversial. Nevertheless, even though the root is embedded in the bone, due to the thermophysical properties of a tooth[36,37], extreme temperatures experienced by the crown will be transferred to the root as well.[38] Besides, a few other studies also reported the use of thermal test on materials placed in the root canals.[39,40]

Numerous *in-vitro* studies have been carried out to evaluate the sealing ability of endodontic sealers using different techniques such as dye leakage, bacterial culture, glucose penetration, and fluid filtration methods.[5,14-16] Glucose penetration method was used in the present study due to the small glucose molecular size which resembles bacterial toxic products, high sensitivity, and it provides a more precise quantitative measurement with fewer operator errors.[19,41,42] Dye leakage study is no longer undertaken largely because the assessment of dye penetration using longitudinal tooth sectioning method ended up with dye dissolution problems and a lower probability of cutting through the deepest part of the dye leakage due to the random selection of cutting axis.[43] Although the bacterial leakage method closely approximates the real clinical situation[5], but due to the antibacterial property of endodontic sealers[8,13], this method might affect the results of a leakage study. A negative control group is crucial in sealing ability test because it can enhance the internal validity of such study by ensuring that a proper baseline of glucose penetration has been achieved. Without a negative control group, it is difficult to hypothesize that the glucose penetration value will start from 0 mmol/L which causes the results obtained to be not reliable.

Unfortunately, most laboratory leakage models are poorly designed and not well-controlled with several confounding factors that reduce the reliability of the results. One of the major factors is most leakage studies used nonpaired extracted teeth with extremely large anatomical root canal variation.[18] Utilizing well-balanced groups with matching canals is still scarce in leakage studies. Hence, the present study used contralateral teeth from the same individual and matched the root canal anatomy of these teeth with strict screening procedures to reduce the bias of different root canal morphology on the results and provide better comparability. To increase the validity and quality of this research work from a previous similar study[7], the present study took patients' age and ethnic origin factors into account since these factors might partially affect the root canal anatomy of contralateral teeth.[18,44,45] Apart from that, results from this study revealed no significant difference in the concentration of glucose penetration when comparing each pair of contralateral teeth. This showed a high level of sensitivity and valid outcomes, thus, creating more concrete evidence to support the reliability of the present methodology. So, the second null hypothesis was accepted.

Additionally, results obtained in *in-vitro* studies might not be appropriate to be directly extrapolated to clinical situations due to the lack of simulated periodontal ligament and the absence of other clinical parameters. However, this study provideda reproducible outcome that can be used for future comparison with various endodontic sealers. Therefore, *in-vivo* studies and clinical trials need to be done to provide more reliable and valid outcomes.

CONCLUSIONS

Within the limitations of this study, it can be concluded that bioceramic sealers demonstrated excellent sealing ability, especially after ageing as compared to resin and calcium hydroxide based sealers. The sealing ability of endodontic sealers decreased as the number of thermocycles increased. Glucose penetration test using matched-pair contralateral teeth after strict radiographic examination provided a well-balanced experimental group.

ACKNOWLEDGEMENT

The authors received financial support from Universiti Sains Malaysia under the short-term grant scheme 304/PPSG/6315195 and 304/PPSG/6316456. The authors thank MrMohd Yusof Soon Abdullah for helping in thermal cycle procedures, Miss Ng Wen Yun for assistance in photo-taking during the project. Furthermore, we acknowledge the management of Hospital Universiti Sains Malaysia (USM), Kubang Kerian, Kelantan, during the process of conducting this research and Septodont for providing the BioRoot RCS.

CONFLICTS OF INTEREST STATEMENT

The authors declare no conflict of interest in this study.

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